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Drosophila Gustation: A Question of Taste

Minireview

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When you sit down for a nice meal and take that first delicious bite, the sensation you experience results from integration of a complex mixture of thermosensation, texture, taste, and smell. The contribution of smell to the sensation of taste is demonstrated by the blandness of food when you have a head cold or by the childhood trick of holding one's nose when eating liver or brussel sprouts. Yet vertebrate odor receptors are not expressed in the tongue, and gustatory receptors are not expressed in the nose (as far as we know!). What are the mechanisms that mediate these complex chemical senses, and are they different because they express different receptor families (for volatile verses nonvolatile ligands) or because the neurons from the two systems connect to the brain in different ways? In a manuscript published in *Cell* this month from Richard Axel's group (Scott et al., 2001), the spatial expression patterns and axonal targets of *Drosophila* neurons expressing the same putative gustatory receptors are examined. They find members of this family expressed in small subsets of cells in the gustatory organs, but surprisingly found some members expressed in the olfactory organs. In one case, a receptor is expressed in a taste organ in the adult and in an olfactory organ in the larvae! These results suggest that in the fly, the difference between taste and smell resides in the neuron in which the receptor is expressed rather than on the class of receptor gene.

The ability to detect and respond appropriately to relevant cues in the environment is a universal feature of living organisms. In animals, the olfactory and gustatory systems are bombarded with a diverse array of chemical signals. Animals have evolved receptor repertoires that ensure detection of the cues relevant to their existence and reproduction. Chemical senses mediate the identification of food and receptive mates and warn of the presence of predators, spoiled food, and noxious environments. The adaptive value of chemical discrimination and associative learning are well recognized. Yet, the neural mechanisms responsible for converting chemical detection into the perception of taste and smell, and ultimately into an appropriate behavioral response, are poorly understood and remain an important question in neuroscience.

Drosophila is proving to be a useful model system to study the link between chemical senses and behavior. Recent studies indicate that general mechanisms mediating odor discrimination are conserved between vertebrates and insects. In *Drosophila*, olfactory cues are detected by neurons located inside sensory hairs or "sensilla" on the antenna and maxillary palp. A family of ~70 seven-transmembrane receptors called ORs (*Drosophila* odorant receptors) have been identified in the fly genome that are thought to mediate odorant responses (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999). As seen in vertebrates, analysis of the axonal projections of *Drosophila* olfactory neurons expressing the same OR reveals that these neurons converge to a single glomerulus in each antennal lobe (the insect equivalent of the olfactory bulb) (Ressler et al., 1994; Vassar et al., 1994; Gao et al., 2000; Vosshall et al., 2000). Therefore, the fly brain can determine if a particular OR has been activated in the peripheral olfactory organs by monitoring neuronal activity in the glomerulus innervated by the neurons expressing that OR. Are similar mechanisms used in insects to convey gustatory information in the fly?

Little is known about the neuronal mechanisms mediating taste in flies. Tanimura's laboratory in Fukuoka, Japan has identified the only confirmed *Drosophila* taste receptor, encoded by the *tre1* gene (Ishimoto et al., 2000). This seven-transmembrane receptor is required for normal sensitivity to the sugar trehalose. The *tre1* receptor is a member of a small gene family consisting of only a few members. This family alone is unlikely to mediate all taste responses. A much larger receptor family expressed in gustatory neurons but not yet proven to mediate taste was identified in John Carlson's laboratory at Yale University (Clyne et al., 2000). These authors identified the GR (gustatory receptor) family using a computer algorithm to probe a database representing 60% of the *Drosophila* genome and identified 23 full-length and 20 partial GR sequences. RT–PCR experiments with 19 GR genes indicated 18 members of this family were expressed in the proboscis (Clyne et al., 2000). However, in situ hybridization experiments, which can readily detect *Drosophila* odorant receptors in the antenna, failed to detect gustatory receptor expression in the gustatory organs. Evidence that these receptors might be taste receptors came from the analysis of mutants defective for the homeotic mutant *pox-neuro* (Clyne et al., 2000). In these mutants, gustatory chemosensory neurons are transformed into mechanosensory neurons (Awasaki and Kimura, 1997). When the Carlson group performed RT–PCR analysis for GR expression in *pox-neuro* mutants, all but one of these receptors was absent. These data indicated most of the GRs were expressed exclusively in gustatory neurons consistent with a role in mediating taste.

Now Scott et al. (2001) have revealed the spatial expression patterns of several members of the GR family. This work makes several contributions to our understanding of GRs and chemosensation in *Drosophila*. First, by scanning the complete genome, a total of 56 GR genes have been identified, including 13 new genes not previously reported. Second, some GR receptors are expressed in different subsets of gustatory neurons in the adult and larval taste organs consistent with a

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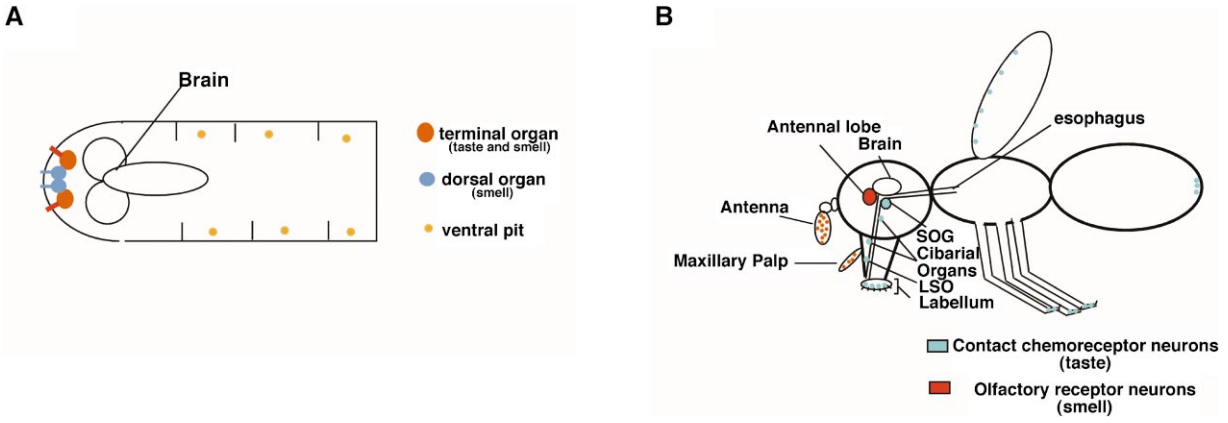

Figure 1. Location of Chemoreceptors in *Drosophila* Larva and Adult Animals

(A) Schematic diagram of the head region of a third instar larva showing the location of the terminal organs, the dorsal organs, and the ventral pit organs.

(B) Schematic diagram of an adult fruit fly showing the location of the olfactory and gustatory organs. SOG, subesophageal ganglion; LSO, labral sense organ.

role in detecting tastants. Surprisingly, some GR receptors are expressed specifically in the olfactory organs, where they probably mediate a subset of olfactory responses. Third, Scott et al. have begun to explore the synaptic targets of chemosensory neurons expressing the same GR receptor in larvae. Finally, they show chemosensory neurons in different larval peripheral organs can express the same GR and that these neurons are wired to different regions of the brain. This raises the possibility that the same stimulus could produce different behavioral responses depending on where the stimulus is perceived. While no ligands are known for specific GR receptors, and therefore the true functional significance of GR expression is speculative, these experiments nevertheless provide a glimpse into the functional organization of gustatory neurons in *Drosophila*.

In contrast to olfactory neurons that are clustered in the antenna and maxillary palp organs, gustatory sensory neurons are distributed in contact chemoreceptors over the entire surface of adult fruit flies (reviewed in Stocker, 1994). Taste receptor neurons are located in sensilla present on the labellum (the tip of the extendable proboscis or mouth), inside the pharnx in the labral sense organ and cibarial organs, along the wing margin, at the tips of the legs, and in the female vaginal plates (Figure 1B). In *Drosophila* larvae, volatile odorants are detected by neurons that reside in the dorsal organ, while gustatory responses appear to be mediated by neurons in the terminal organ, in chemosensory neurons located in the ventral pits present on each thoracic hemisegment (Figure 1A), and some of the neurons in the dorsal organ. None of the OR family members are expressed in these gustatory organs. However, the recently described GR family of receptors may be taste receptors expressed by these gustatory neurons.

There Are at Least 56 GR Members

Scott et al. have used the complete genome sequence to expand the GR family to 56 members. All have a 33 amino acid region of similarity in the seventh transmembrane region that defines them as GR members. However, 33 of the 70 ORs expressed in the antenna and maxillary palps also show similarity to this GR motif, although the similarity is weaker in the ORs. Why this particular region is conserved more than any other region is a mystery but suggests that the OR and GR families may have diverged from a common receptor family. It should be noted that 56 receptors is probably a low estimate because some genes are alternatively spliced to produce more than one receptor (Clyne et al., 2000), and it is possible that additional members will be identified in the genome that lack or have a divergent 33 amino acid motif.

In Situ Hybridization Analysis Detects GR Expression in Taste and Olfactory Organs

Scott et al. performed in situ hybridization experiments on adult head tissue sections using each of the 56 GR members as probes. For nearly all the GR genes, no signal was detectable. This probably reflects low-level expression for most of these genes, as they are detectable in the head with RT–PCR. However, for nine GR members hybridization signals were observed. Consistent with a role in taste transduction, six of the GR genes were expressed in discrete subpopulations of neurons in the adult proboscis. Surprisingly, three were expressed in topographically restricted olfactory neurons in the antenna. Indeed, as is observed for olfactory neurons expressing the same OR, antennal neurons expressing the same GR project to a single glomerulus in the antenna lobe, suggesting these neurons are functionally related. These expression patterns imply that different members of the GR family mediate olfactory as well as gustatory responses.

To more precisely define the cells expressing individual GR receptors, the upstream regulatory sequences from several GR receptors were used to drive transgene expression. Fifteen GR promoters were cloned upstream of the Gal4 yeast transcription factor gene, and these constructs were placed into transgenic flies carrying UAS-LacZ or UAS-GFP reporter genes regulated by the Gal4 gene. Five of these promoters expressed reporter products in subsets of taste receptor neurons in the labellum (GR22b1, 28A3, 32D1, 47A1, and 66C1).

Interestingly, each of the five GR promoters express a reporter in a few sensilla, and only one of the four rows of sensilla on the labellum is positive for any given promoter. This is reminiscent of vertebrate odorant receptors that are expressed within one of four zones in the olfactory epithelium (Ressler et al., 1993). Furthermore, only one of the four chemosensory neurons within the sensillum expressed the transgene. Of the five GR promoters that express in the labellum, three are also expressed in the pharyngeal taste organs, and one of these is also expressed in gustatory neurons in the feet, indicating that a single receptor can be expressed in diverse chemosensory organs in the adult animal. When flies carrying two GR promoter transgenes were created, the reporter pattern was additive, indicating these GR genes are not coexpressed in the same neurons. Importantly, this indicates that *Drosophila* gustatory neurons express different complements of receptors. However, because so few GR members have been mapped, it is not possible to determine if these neurons express a single GR member or just different complements.

In larvae, five GR promoters drive transgene expression in gustatory neurons localized to the terminal organ, where most neurons mediating taste reside in the larva. Typically there is a single, bilaterally symmetric neuron labeled in each larva. Interestingly, one GR promoter (GR2B1) drives reporter expression exclusively in a single cibarial taste neuron in the adult but is expressed in several neurons in the larvae including two neurons in each dorsal organ, one neuron in each terminal organ, and in one neuron in each of the ventral pits along the front half of the larval body. Therefore, a single receptor can be expressed in different gustatory organs in both adults and larvae.

Axonal Projections of Neurons Expressing the Same GR

In the olfactory system, the quality of an odor stimulus is encoded by defined patterns of neuronal activity. Olfactory neurons expressing the same receptor gene project to the same glomerulus in the antennal lobe (Vosshall et al., 2000). Projection neurons relay information about glomerular activity to higher centers where the perception of odor occurs. The mechanisms mediating odor discrimination, therefore, appear to be conserved in vertebrates and insects even though the receptor gene families are not. Are there any similarities between information processing in the gustatory system and olfactory systems in *Drosophila*?

To determine where functionally related gustatory neurons expressing the same putative taste receptor project, the GR promoter-Gal4 transgenic flies were crossed to UAS syb-GFP reporter flies. This reporter links GFP to neuronal synaptobrevin, a synaptic vesicle protein. When expressed in neurons, this reporter localizes GFP to the presynaptic terminals, which allows visualization of the targets of the neuron (Estes et al., 2000). This approach was used successfully to map the glomerular targets of olfactory neurons expressing the same OR gene (Vosshall et al., 2000). Scott et al. looked at the projections of gustatory neurons in the more accessible larvae to identify the axonal targets of these neurons. Three receptors expressed in the larvae were examined in detail (Figure 2). Promoters for GR receptors GR32D1 and GR66C1 were crossed to the UAS syb-GFP reporter, and the neurons expressing these transgenes were found to project to the subesophageal ganglion (SOG) located in the anterior part of the larval hindbrain. A terminal arborization was present for each that was more diffuse and elongated than is observed for a glomerulus in the antennal lobe. Interestingly, when both promoters were crossed into the same fly, a larger region of the SOG was GFP positive, indicating that neurons expressing the two different receptors project to slightly different but overlapping regions of the SOG. It is not clear if any SOG neurons receive input from both populations of neurons. The answer to this question must await a higher resolution analysis that could be done for example by directly fusing the GR32D1 and GR 66C1 promoters to different colors of GFP.

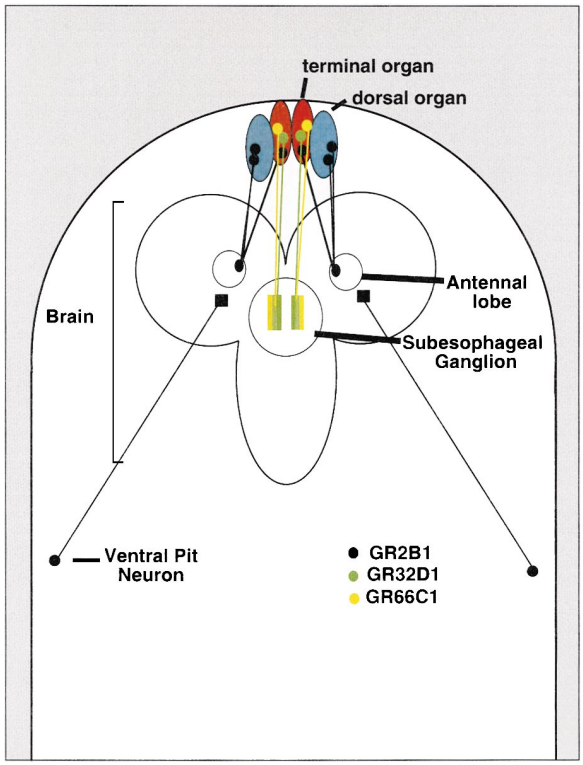

Figure 2. Schematic Diagram of the Head of a Third Instar Larva Depicting the Neurons and Projections of Chemosensory Neurons Expressing Specific Gustatory Receptors

GR2B1 neurons and projections are shown in black, GR32D1 expressing neurons and projections are shown in green, and GR66C1 neurons and projections are shown in yellow. Note overlap in projections of GR32D1 and GR66C1 neurons in the subesophageal ganglion and distinct projections of GR2B1 neurons located in ventral pits verses those in the terminal and dorsal organs.

Finally, the GR2B1 promoter that drives reporter expression in one terminal organ cell, two dorsal organ cells, and in the ventral pit organs had interesting projections worth noting. Larval neurons expressing this receptor project to the antennal lobe where odor responses are thought to be processed. However, axons from the ventral pit chemosensors projected to a region posterior to the antennal lobe. Therefore, the same receptor can

be expressed in chemosensory neurons located in different areas and project axons to different sites in the brain. The same stimulus, therefore, could produce distinct behavioral outputs, depending on which neurons have been activated. Furthermore, GR2B1 is expressed in a gustatory organ in the adult (the labral sense organ) and in neurons located in the larval olfactory organ with projections to the antennal lobe, suggesting the same receptor may mediate both olfactory and gustatory responses! It will be interesting to identify the ligand(s) of this receptor to determine if they can function as both odorants and tastants.

Is there a spatial representation of tastants in the subesophageal ganglion like that seen in the olfactory system? The synaptic arbors of larval gustatory neurons expressing the same GR appear to innervate a rather long strip in the SOG and are not as spatially restricted as seen, for example, in olfactory neurons converging into glomeruli in the adult. However, the convergence of GR2B1 neurons to the antenna lobes in the larval brain may not be as precise either. Perhaps it is not necessary for the larvae to discriminate chemical cues with the precision required of an adult. Scott et al. do not report the synaptic target patterns of the labeled gustatory neurons expressing the same GR in the adult SOG. It would be informative to know if there is convergence of labellar neurons expressing the same GR in the adults. A better understanding of the representation of taste in the *Drosophila* brain will require additional studies with additional GR members to get a better picture of the synaptic targets of neurons expressing the same GR. This may require improvements in techniques to label cells expressing very low levels of receptor molecules, but no doubt these results will be achieved in the near future.

Drosophila *Gustatory Coding*

What can these data tell us about the mechanisms of gustatory coding in *Drosophila*? With only a handful of GR-expressing neurons mapped, any conclusions are speculative. However, different GRs are expressed in small subsets of gustatory neurons, indicating that different taste neurons express different complements of receptors and thus are likely to be tuned to different tastants. This molecular data is contradictory to previous electrophysiological studies of *Drosophila* labelum sensilla that concluded that each of the chemosensory neurons in each sensillum was tuned to a different category of taste and that all the sensilla were identical. To resolve these contradictions, it will be necessary to define the biological roles of GRs. For example, identifying GR mutants that correlate with taste defects, or identifying ligands that activate specific GRs, will permit more detailed studies to address this issue.

Relevance to Vertebrate Gustation?

In vertebrates like ourselves, the sensation of taste is an extraordinarily complex phenomenon that simultaneously integrates information about food texture, temperature, and smell in addition to the classic taste qualities of sweetness, bitterness, sourness, saltiness, and unami (the meaty taste of glutamate). Taste receptor cells are concentrated in taste buds located on papillae on the tongue and soft palate. Taste buds arise from epithelial cells (not neurons) and thus developmentally are quite different from the gustatory receptor cells in *Drosophila*. Individual taste receptor cells that detect bitter compounds appear to express multiple receptors, which may explain in part why diverse chemical structures have the same bitter taste quality (Adler et al., 2000). However, taste cells that respond to bitter compounds only respond to subsets of bitter tastants, suggesting that taste cells may express a more limited repertoire of taste receptor proteins (Caicedo and Roper, 2001). This may turn out to be similar to the situation in *Drosophila* taste neurons. Recording from individual vertebrate gustatory neurons (that innervate multiple taste cells in multiple taste buds) reveals that they carry information about multiple taste modalities (like sweet, salt, or acid; reviewed in Smith and St. John, 1999). Therefore, it is likely that individual vertebrate gustatory neurons contribute to multiple taste qualities, and taste is determined by the relative activity in populations of gustatory neurons. It remains to be seen if similar mechanisms operate in the fly, but given the remarkable conservation in olfactory mechanisms between flies and vertebrates, flies may indeed turn out to be a very tasty model system indeed.

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